N:P RATIO OF SYNTETIC WASTEWATER IMPACT UPON CHANGING ON SPECIES, GROWTH RATE, CHEMICALCOMPOSITION OF MICROALGAE: BATCH CULTURE TECHNIQUE

*Nita Rukminasari**

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ABSTRACT

The aim of this study to examine the effect of N:P ratio of synthetic wastewater on changing species composition, growth rate, carbohydrate content of microalgae. Microalgae was cultured for 14 days at 1 L round flask bottles using batch culture with WC media and 24 hours light illumination. Nitrogen were consists of 5 concentrations, such as 10, 20, 30, 40 and 50 mg/L-N and for Phosphorus were used 5 concentration which were 2, 4, 6, 8 and 10 mg/L-P. Variables measured were growth rate based on optical density, species composition, total fatty acid and carbohydrate content. There were 11 genus of algae found, which were *Chlorella sp, Oscillatoriasp, Scenedesmussp, Actinastrumsp, Chlorococcumsp, Coelastrumsp, Gleokininasp, Keratococcussp, Merismopediasp, Monoraphidiumsp and Spirulina sp*. Three species dominated at all NP treatments, such as *Oscillatoriasp, Chlorella sp* and *Scenedesmus sp.* Samples with low P concentration were dominated by *Oscillatoriasp*, while treatments with high P concentration were dominated by *Chlorella sp* and *Scenedesmus sp.* The highest total cell abundance was found at treatment of 40 mg/L-N and 6 mg/L-P, account for 2,272,500 cell/mL. However, there was no particular pattern of total cell abundance among NP treatments. In general, specific growth rate was slightly low at high P concentration for all N treatments. There was no clear pattern of % carbohydrate per dry weight among NP treatments. The highest % carbohydrate per DW was 1.16% at lowest N concentration (10 mg/L-N) and at the highest P concentration (50 mg/L-P). Treatment with 30 mg/L-N and 6 mg/L-P showed having the lowest % carbohydrate per DW.

Keywords : N:P ratio, synthetic waste water, microalgae, batch culture technique.

INTRODUCTION

The most frequent limiting nutrients for primary production in marine, freshwater, and terrestrial ecosystems was N and P (Hecky and Kilham 1988, Downing 1997 and Elser et al, 1990). Variability in N:P stoichiometry of primary producers was determined by the variation in N:P supply ratios (Hall 2009). In contrast to the plasticity of phytoplankton stoichiometry, the stoichiometry of consumers exhibits less variability, resulting in a mismatch between C:N:P stoichiometry of primary producers and consumers (Elser et al. 2000a, Andersen et al. 2006).

Resource-ratio theory, first developed for phytoplankton (Tilman 1977, 1982, 1985), predicts that changes in the environmental ratios of two essential nutrients will cause changes in plant community structure due to exploitative competition among taxa with different optimal nutrient ratios. Two species with different optimal nutrient ratios are able to coexist only if each species is a better competitor for the nutrient most limiting to the other species.

The success of phytoplankton species in natural communities depends on whether the cellular growth rate exceeds or equals loss rates from dilution, sedimentation, physiological death, and grazing (Hecky and Kilham 1988). Nutrient limitation of growth rates is important for both the stoichiometry of phytoplankton biomass and the determination of phytoplankton community structure (Terry et al. 1985b, Sterner and Elser 2002, Flynn et al. 2002).

Competition for limiting nutrients is seen as an important factor in the determination of phytoplankton community composition (Tilman et al., 1982; Sommer, 1989a; Grover, 1997). Tilman's

* **Korespondensi:**
Marine Science and Fisheries Faculty, Hasanuddin University
Jl. Perintis Kemerdekaan Km. 10, Universitas Hasanuddin
Makassar, 90245 email: nita.r@unhas.ac.id; nitasari_02@hotmail.com.

resource competition theory states that under nutrient limitation in equilibrium conditions, those species, which have either the lowest requirement for the limited resource or the high- est ability to utilize it, will succeed in competition (Tilman, 1977, 1982; Tilman et al., 1982). The ability of the algae to compete for nutrients is determined by its physiological properties, e.g. half saturation constants, growth rate, transport rates and storage capacities (Flynn, 2002).

Nutrient ratios influence the growth, physiological state, and community structure of phytoplankton. Shifts in N:P weight ratios from 10 to 5 have resulted in the decline of total algal biomass (Pickney et al,2001). Egge and Heimdal (1994) showed decrease in diatom population when the N:P increased. In N:P weight ratios of 14.5, 29, and 58 diatoms out-competed *Phaeocystis* in enclosure experiments (Escaravage et al. 1996). A shift from a Microcystis-dominant to Oscillatoria-dominant phytoplankton community was associated with the change in the prevailing N:P of the water as reported by Tsujimura and Okubo (1999) for Lake Kasumigaura in Japan. Reduction in *Microcystisaeruginosa* population was also reported as a result of the elevation of the total N to total P ratio TN:TP (Zohary et al. 1996). Kahruet al (1999) reported that the N-fixing *Aphanizomenonflos-aquae* bloomed in hypertrophic lakes only when the TN:TP ratio was less than 16, otherwise, the lakes were dominated by *Planktothrixagardhii*. N-P ratios also affect algal growth, cell composition and nutrient uptake on the individual species level.

Nowday, aquatic ecosystem have a serious threat for overloading of nutrient especially N and P from the domestic, agriculture and industrial wastewater. Consequently, the loading of nutrient from wastewater affected significantly to the ratio of macronutrient (N, P, and C) in the waters. The major effect of releasing wastewater rich in organic compounds and inorganic chemicals, such as phosphates and nitrates, is the eutrophication of freshwater ecosystems (McGinn et al, 2012 and Mulbry et al, 2008).

The composition of wastewater is a reflection of the life styles and technologies practiced in the producing society (Gray, 1989). It is a complex mixture of natural organic and inorganic materials as well as man-made compounds. Three quarters of organic carbon in sewage are present as carbohydrates, fats, proteins, amino acids, and volatile acids. The inorganic constituents include large concentrations of sodium, calcium, potassium, magnesium, chlorine, sulphur, phosphate, bicarbonate, ammonium salts and heavy metals (Hori, 2002; Lim et al., 2010).

Microalgae have high potential to remove inorganic nutrients from the wastewater and to yield a biomass useful to produce biofuels, fertilizers or other bioproducts. Algae growth requires the availability of primary nutrients, such as carbon, nitrogen and phosphorus, and of micronutrients, which can be costly if they need to be added in great amounts (Christenson and Sims, 2011); to this end the use of wastewater can reduce the cost of algae production minimizing the addition of nutrients (Samori et al, 2013). They also explained that microalgae assimilate inorganic nutrients and release oxygen through the photosynthetic process; on the other hand free oxygen is essential to allow efficient bioremediation of organic compounds by heterotrophic bacteria which, in turn, produce carbon dioxide (CO2) utilized by microalgae for photosynthesis. Ogbhna et al (2000) explained that a major advantage of using photosynthetic microorganisms for wastewater treatment is the possibility of combining wastewater treatment with production of useful metabolites.

Most of the studies on the use of microalgae for wastewater treatment have been based on the use of a monoculture to remove a specific nutrient (mainly nitrogen or phosphate) and only a few studies have been reported on the use of mixed algal cultures for wastewater treatment (Gantar et al., 1991). None of study have been conducted to examine N:P ratio of synthetic wastewater impact upon changing of species composition, growth rate, chemical composition of microalgae and nutrient removals.

The aim of this study will be examine the effect of N:P ratio of synthetic wastewater on changing species composition, growth rate, chemical composition of microalgae and nutrient removal by natural species of microalgae.

MATERIAL AND METHODS

Microorganisms and N:P ratio treatments

Microalgae will be collected from artificial lake, which is located at KU Leuven, Kulak Campus. Natural species of microalgae will be stored at refrigerator for 14 days before the experiment starting. Initial species composition will be determined using inverted microscope with microalgae identification guide books. Microalgae will be identified till genus level. Microalgae will be cultivated for 14 days at 1 L round flask bottles using batch culture with 24 hours light illumination.

The medium for microalgae cultivation will be used WC medium without N and P compound. For Nitrogen will be consists of 5 concentrations, such as 10, 20, 30, 40 and 50 mg/L-N and for Phosphorus will be used 5 concentration which were 2, 4, 6, 8 and 10 mg/L-P. There will be 25 combination of N:P ratio.

Measurement of cell growth and species composition

The culture will be monitored daily by optical density measurement at wavelength of 750 nm using Spectrophotometer DR 2800 HACH LANGE.
Biomass productivity (gL^{-1} d^{-1}) will be calculated through the variation in biomass concentration (gL^{-1}) within a cultivation time (d) according to the following equation: $P =[X_1 - X_0]/ [t_1 - t_0]$ where X1 and X0 were the biomass concentrations ($g\ L^{-1}$) on day t1 and t0, respectively.

Specific growth rate **μ** (d^{-1}) will be calculated from the following equation :

$$\mu = \ln (OD1/OD0)/[t1 - t0]$$

where OD1 and OD0 were the optical absorbances at a wave- length of 750 nm on day t1 and t0, respectively.Species composition changing at every N:P ratio treatments will be determined at the end of culture period.

Determination of carbohydrate content

Carbohydrate were extracted from cultures following Moheimani et al (2013). Dried samples (10 mg) were sonicated on intensity 20 for 1 min. 2 mL of H2SO4 2M was added to 2 mL of sonicated sample. The solution was put in hot water bath at 100oC for 1 hour. After cooling for 10 mins to room temperature, the solution was centrifuged for 10 mins on 2000 g and let it sedimented over night at cool room. The total carbohydrate content of microalgae was determined by the Phenol Sulfuric Method (Dubois et al, 1956 and Cuesta et al, 2003) using glucose as standard.

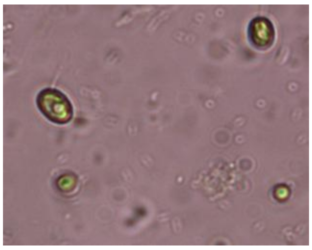
Data analysis

The mean and SE will be calculated for each treatment from three independent replicate cultures and will be graphed. Student's t-test will be performed to compare the test group with the controls.

RESULTS AND DISCUSSION

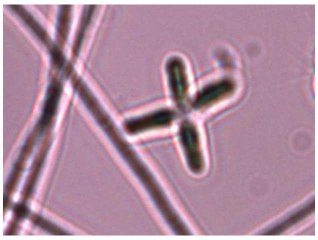
Species composition and cell abundance

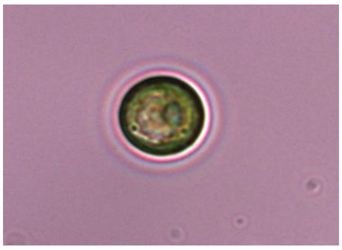
There were 11 genus of algae found, which were *Chlorella sp, Oscillatoriasp, Scenedesmussp, Actinastrumsp, Chlorococcumsp, Coelastrumsp, Gleokininasp, Keratococcussp, Merismopediasp, Monoraphidiumsp and Spirulinasp*(Figure 1). Three species dominated at all NP treatments, such as *Oscillatoriasp, Chlorella sp* and *Scenedesmus sp*. Samples with low P concentration were dominated by *Oscillatoriasp*, while treatments with high P concentration were dominated by *Chlorella sp and Scenedesmus sp.* Most of microalgae that found were from Chlorophycea class.


Chlorella sp

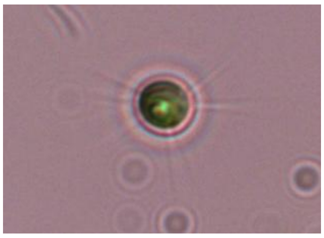

Oscillatoria sp

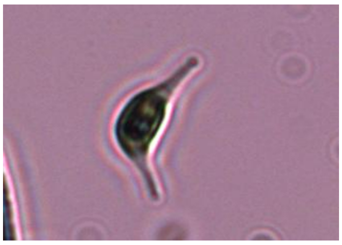

Scenedesmus sp

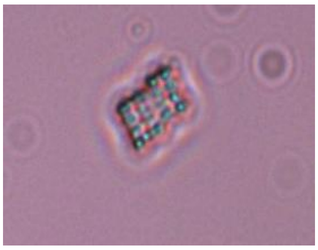

Actinastrum sp


Chlorococcum sp


*Coelastrum*s


Gleokinina sp


*Keratococcus*sp


Merismopedia sp


*Monoraphidium*sp


Spirulina sp

Figure 1. Microalgae species were found at the water samples from the articificial lake.

The highest total cell abundance was found at treatment of 40 mg/L-N and 6 mg/L-P, account for 2,272,500 cell/mL (Figure 2). However, there was no particular pattern of total cell abundance among NP treatments. Jeppesen*et.al*, (1997) also support this study and they found that phytoplankton assemblages structure changes with Total Phosphate concentration in the Northern Hemisphere temperate lakes; cyanobacteria dominate in water bodies at intermediate level of TP (0.1-0.5 mg/L), green algae dominate at hypertrophic levels and a mixed community dominates at TP levels < 0.1 mg/L. We also measured length of *Oscillatoria* filament for each NP treatment. We found that length of *Oscillatoria* filament increased with increasing N concentration. In the contrary, the length of filament decreased with increasing P concentration.

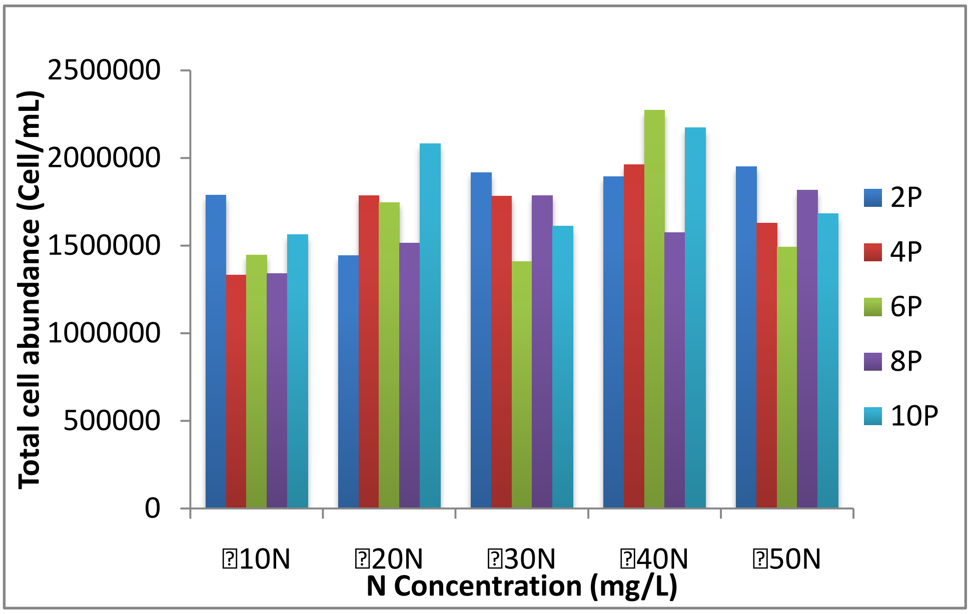


Figure 2. Total cell abundance at different N:P ratio.

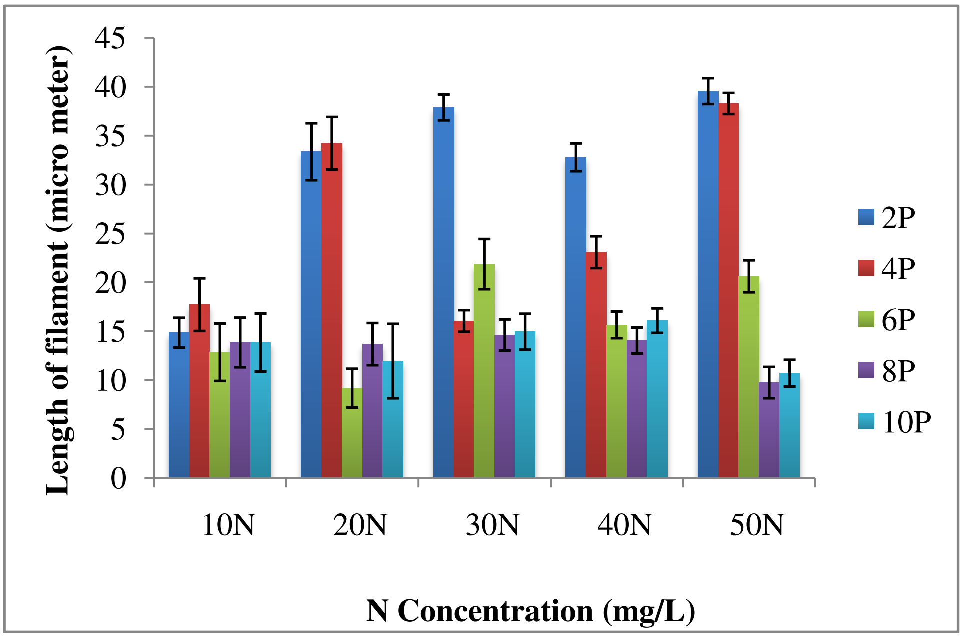


Figure 3. Length of filament Oscillatoriasp at different N:P ratio (X ± SE, N = 30).

Growth rate

The highest specific growth rate was 0.336 cell/day for 20 mg/L-N and 2 mg/L-P treatment and treatment with 50 mg/L-N and 10 mg/L-P had the lowest specific growth rate, account for 0.221 cell/day (Figure 4). In general, specific growth rate was slightly low at high P concentration for all N treatments.

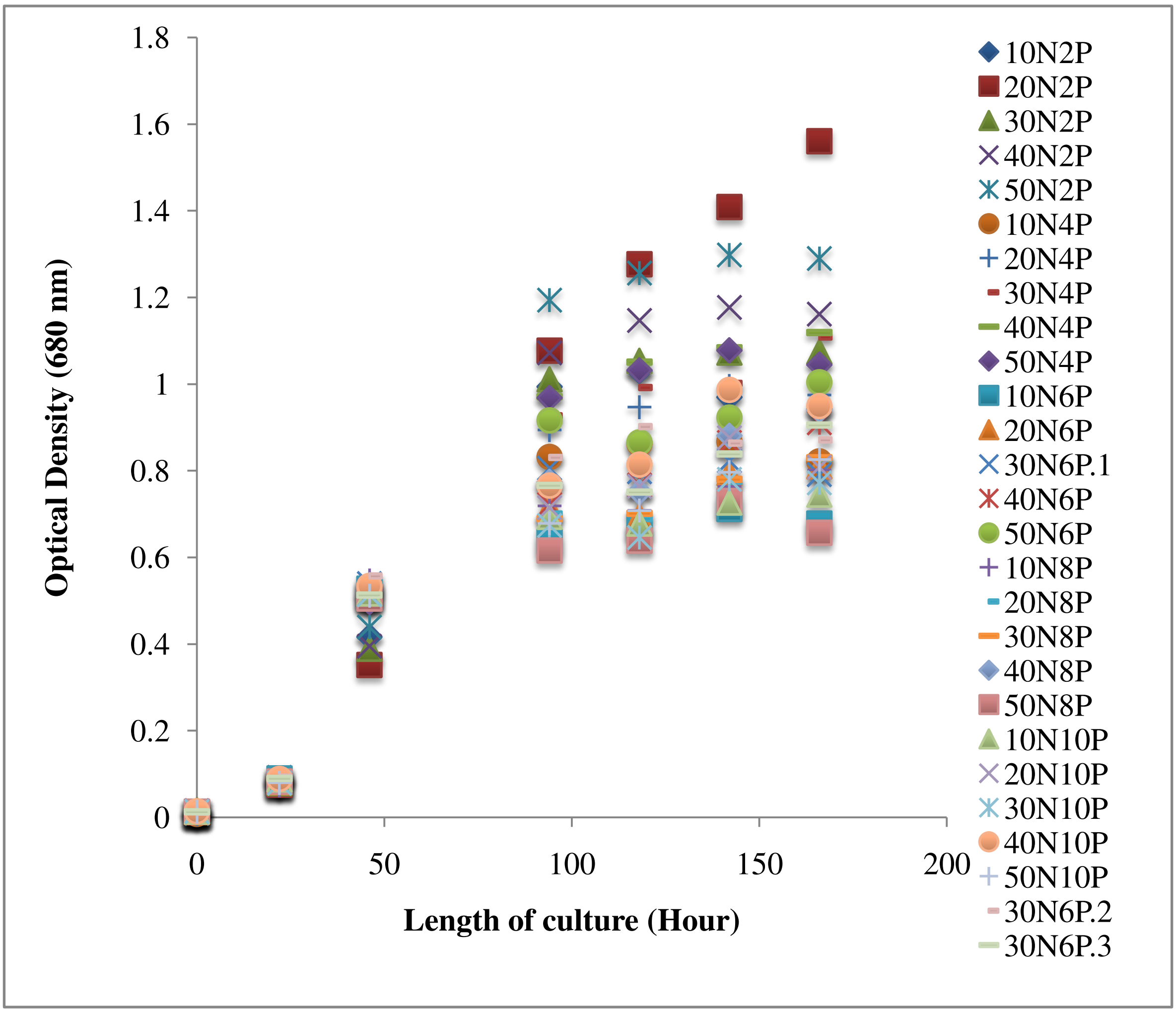


Figure 4. Growth rate based on optical density 680 nm of different N: P ratio

Carbohydrate content

There was no clear pattern of % carbohydrate per dry weight among NP treatments. The highest % carbohydrate per DW was 1.16% at lowest N concentration (10 mg/L-N) and at the highest P concentration (50 mg/L-P) (Figure 5). Treatment with 30 mg/L-N and 6 mg/L-P showed having the lowest % carbohydrate per DW.

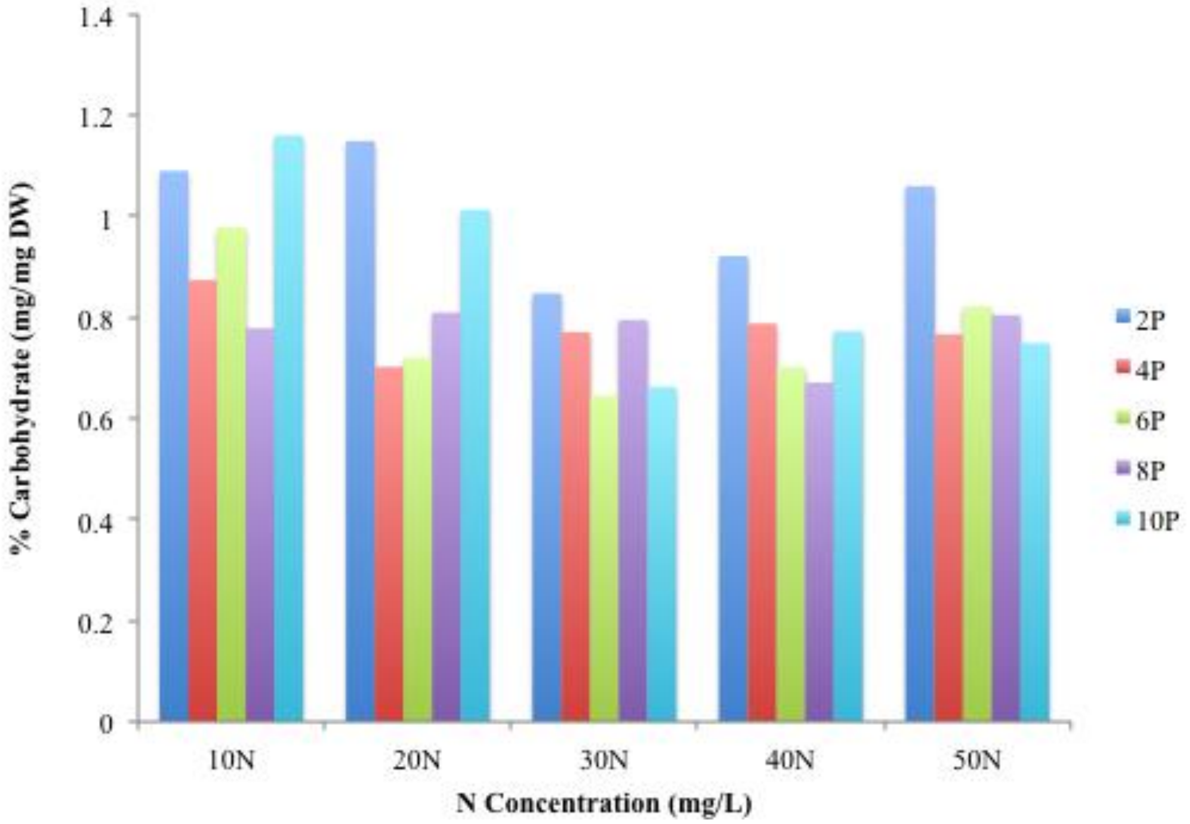


Figure 5. % Carbohydrate (mg/mg DW) at different N:P ratio

CONCLUSIONS

- NP ratio impacted significantly on shifting of species composition and changing in length of Oscillatoria filament.

- Specific growth rate of mixed algae was slightly affected by P concentration. However, in general NP concentration was not effect on specific growth rate.

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